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WO 02/45711 A1

(54) Title: USE OF ANTI-MUSCARINIC AGENTS AND CALCIUM CHANNEL BLOCKERS IN COMBINATION

(57) Abstract: An anti-muscarinic agent and a calcium channel blocker are suitable for simultaneous, sequential or separate use in the treatment of a muscle tone disorder or a proliferative, inflammatory or secretory condition.

USE OF ANTI-MUSCARINIC AGENTS AND CALCIUM CHANNEL BLOCKERS IN COMBINATION

Field of the Invention

This invention relates to the use of anti-muscarinic agents and calcium channel blockers in combination.

Background of the Invention

Atropine is a very well established anti-muscarinic agent. The drug exhibits all the pharmacological and toxicological effects of this class of therapeutic agents. The drug itself is a potent bronchodilator, acting on the peripheral smooth muscle lining of the bronchi. In addition, the drug is known as an antisialogogue, being used to dry mucous secretions, as well as having central effects as exemplified by its use in motion sickness, and in schizophrenia. The ubiquitous nature of the muscarinic receptors and the indiscriminate nature of the binding of atropine to these receptors are also responsible for its side-effects. Side-effects include constipation, urinary retention, confusion, blurred vision and dry mouth.

Muscarinic antagonism is utilised in the treatment of respiratory disease. A number of successful therapies are based upon the bronchodilatory and antisecretory properties of anti-muscarinic agents.

Direct evidence of anti-proliferative activity by muscarinic antagonism comes from the demonstration by Qiu *et al* (Sheng Li Hsueh Pao 1995, 275-80) that T lymphocytes undergo enhanced proliferation through the mediation by acetylcholine, and that atropine abolishes this effect completely.

The clinical utility of anti-muscarinic therapy remains limited, due to systemic side-effects. These can be managed by local administration to diseased tissue. Such a mode of administration occurs with the bronchodilator ipratropium when used in inhaled form. Alternatively, modifications to receptor selectivity can be considered, for example the use of selective M3 antagonists in asthma. However, both of these approaches have major drawbacks either in terms of poor tissue accessability or persistent side-effects.

WO-A-98/00119 discloses the use of agents that affect non-neuronal acetylcholine functions, for the treatment of skin ailments. It also discloses that

topically effective antagonists of muscarinic receptors, including ipratropium, are useful for the treatment of skin ailments. Various skin ailments that are disclosed include atopic dermatitis, neurodermatitis, psoriasis and cholinergic urticaria.

WO-A-01/10427 discloses that skin conditions are treated by the topical application of a quaternary ammonium or other compound having anti-muscarinic activity, a high dipole moment (greater than 4D) and high anti-proliferation activity (at least 50% inhibition at 10 μM). It may also have high receptor-binding activity (half-life for receptor dissociation greater than 0.11 h at M1).

WO-A-01/76575 discloses a pharmaceutical composition for pulmonary administration, comprising an anti-muscarinic agent. The preferred agent is glycopyrrolate. The compositon is particularly suitable for the treatment of asthma, cystic fibrosis and chronic obstructive pulmonary disease.

Calcium channel blocking has for some time been an attractive approach to the treatment of respiratory inflammatory diseases. However, the available agents have lacked the potency for effective treatment and no effective therapies have been developed.

Summary of the Invention

It has now been appreciated that there is an alternative and attractive method of overcoming the problems described above. According to this invention, calcium channel blockade is used in conjunction with muscarinic antagonists, e.g. those that have long receptor-binding characteristics, to provide both longer duration of action with enhanced effect and the use of lower doses. When administered topically, the local effect of the drug can be significantly enhanced whilst ensuring that systemic and side-effects can be further minimised.

The invention is based on the discovery that the action of calcium channel blocking agents can be potentiated by the combined activity of muscarinic antagonism, and *vice versa*. Using the guinea pig functional model of smooth muscle relaxation, it has been demonstrated that a small addition of an anti-muscarinic agent causes almost a ten-fold increase in effect of the calcium channel blocking agent. This for the first time allows the consideration of

calcium channel blocking agents as components of effective treatments for respiratory disease.

The respective active agents used in the present invention may be formulated together, e.g. in "kit" form. Alternatively, one may be administered to a subject who is undergoing therapy with the other.

Using these agents in combination allows a precise tailoring of activity for most desirable patient outcome. For example, for patients with additional complications in which anti-muscarinic activity gives rise to additional side-effects, a threshold level of anti-muscarinic agent can be used. Such patient groups include those suffering from cardiac complaints, glaucoma and problems with gut motility. In addition, patients suffering from cholinergic load as a result of concomitant medicine will also benefit from bronchial smooth muscle relaxation as a result of minimal but threshold muscarinic antagonism and maximal calcium channel blockade. Conversely, patient groups with severely compromised respiratory function benefit from maximal muscarinic antagonism. The currently available agent (ipratropium) provides only short term maximal antagonism. However, the calcium channel blocking agents are known to be metabolically stable in the lung and in some cases such as verapamil are sequestered and accumulate in lung tissue. The addition of an appropriate calcium channel blocking agent provides substantial background smooth muscle relaxation and less reliance upon precise dosing intervals for best patient benefit.

The use of certain anti-muscarinic components leads to a low plasma half-life, thus reducing their side-effects. The route of administration of these molecules can be used to further limit systemic exposure and side-effects.

Description of Preferred Embodiments

The present invention is particularly suitable to topical administration, e.g. dermally, to the lung, and to the gastrointestinal tract. For dermal use, and as described in WO-A-01/10427, keratinocyte proliferation may be inhibited, using anti-muscarinic agents. Preferred such agents have a dipole moment of greater than 4.0, since they show limited systemic exposure due to poor passage across the skin to the circulatory system.

Anti-muscarinic agents for use in this invention preferably also have high receptor-binding affinity. A long duration of action is extremely desirable for a topically applied drug to treat local conditions. This leads to low reapplication rates of medication, which in turn ensures minimum disturbance to patient lifestyle, and high patient compliance. Compounds with high receptor binding affinity include glycopyrrolate, ipratropium and tiotropium.

At the clinical level, glycopyrrolate is known to have a longer duration of action in muscarinic antagonism than ipratropium; see J. Allergy Clin. Immunol. (1988) 82:115. In addition, in Frey's syndrome, a two day duration of action from a single dermal application appears to be common, in the use of glycopyrrolate.

Disse *et al*, Life Sciences (1993) 52/5-6:537-544, compared the dissociation rates of ipratropium and tiotropium. For muscarinic receptor MI, the half-lives were 0.11 h and 14.6 h; for M3, they were 0.26 h and 34.7 h, respectively. The relatively low off-rate and long half-life for tiotropium are responsible for its very long duration of action in smooth muscle relaxation involving muscarinic antagonism.

More particularly, suitable anti-muscarinic agents for dermal use at least in the invention may initially be identified by the Assay Protocol described in WO-A-01/10427, which is a model of psoriasis and thus of a proliferative skin condition. An agent for use in the invention preferably has an IC_{50} value below 100 μM, most preferably below 10 μM, e.g. below 1 μM, and most preferably below 100 nM.

Anti-muscarinic agents that are suitable for administration to the lung are described in WO-A-01/76575. Examples of anti-muscarinic agents that can be used in the invention include ambutonium, benzilonium, dibutoline, diphemanil, emepronium, glycopyrrolate, isopropamide, lachesine, mepenzolate, methantheline, oxyphenonium, oxytroprium, penthienate, phenthimentonium, pipenzolate, poldine, tiemonium, tiotropium, tricyclamol and tridihexethyl. Glycopyrrolate is preferred.

The calcium channel blockade may be provided by any of a number of agents that are known to those skilled in the art. Examples include diltiazem, verapamil and dihydropyridine drugs. Other such agents will typically have at

least substantially the same (at least 50%) of the activity of the given agents, in an assay for calcium channel blocking activity.

These and other compounds for use in the invention may be provided in the form of a free base or salt. All such forms are within the scope of the invention, and in particular salts, organic and inorganic, are included. For example, quaternary ammonium compounds may be provided as a halide or other salt.

Many compounds suitable for use in the invention exhibit isomerism, whether optical or structural (stereoisomerism/regioisomerism). Such compounds include glycopyrrolate and tiotropium. Application of a single isomer or a non-stoichiometric mixture of isomers, e.g. a non-racemic mixture, in the case of optical isomers, may optimise the desired antiproliferative activity.

This invention is of particular value in the treatment of respiratory conditions, including respiratory inflammation and respiratory proliferation. In particular, the invention may be used in the treatment of chronic obstructive pulmonary disease (COPD), asthma or cystic fibrosis, or associated conditions. For the treatment of each condition, the active agents may be administered by inhalation.

The active agent may also be administered by the oral route. This can be used in therapy where the condition to be treated is a muscle tone disorder, e.g. of the gastrointestinal or urinary tract, or which involves gut motility or urinary incontinence. Here and in general, the anti-secretory and smooth muscle action of the new combination can be utilised.

Dermal conditions that may be treated include all forms of psoriasis, including psoriatic and scalp arthritis, skin cancer, melanoma, pemphigus, atopic dermatitis, neuro-dermatitis, eczema, contact dermatitis, acne, leprosy, seborrheic dermatitis, lupus and urticaria. The invention is particularly suited to the treatment of topical proliferative conditions such as psoriasis. Treatment may be combined with radiological therapy. Alternatively or in addition, treatment may be combined with a conventional agent, of which examples include steroids, vitamins A and D and their analogues, salicylates, anthralines and coal tar preparations.

Conventional topical formulations and administration techniques may be used. For example, for dermal use, suitable compositions include, but are not limited to, creams, ointments, gels, shampoos, lotions, ionotophoresis, patches and emollients. The two active agents may be formulated in a mixture of independently, e.g. in a kit. In particular, this invention provides anti-muscarinic agents to treat skin condition by topical administration, in which the drug is placed in a formulation system in which the drug flux across the skin is maintained at such a rate that systemic blood levels are retained at a low level. However, the drug flux is maintained at a level to effect topical activity in the skin. In this way, anti-muscarinic agents may be used that would otherwise be limited by their side-effects.

Devices and formulations suitable for delivery by inhalation are known to the skilled person. The composition may be prepared for delivery as an aerosol in a liquid propellant, for example for use in a pressurised metered dose inhaler (PMDI's). Propellants suitable for use in a PMDI are known to the skilled person, and include CFC-12, HFA-134a, HFA-227, HCFC-22 (difluorochloromethane), HFA-152 (difluoroethane and isobutane).

In a preferred embodiment of the invention, the compositions are in a dry powder form, for delivery using a dry powder inhaler (DPI). Dry powder inhalers are known. The dry powders for use in the inhalers will usually have a mass medium aerodynamic diameter of less than 30 μm, preferably less than 20 μm and more preferably less than 10 μm. Microparticles having aerodynamic diameters in the range of 5 to 0.5 μm will generally be deposited in the respiratory bronchioles, whereas smaller particles having aerodynamic diameters in the range of 2 to 0.05 μm are likely to be deposited in the alveoli.

The microparticles may also be formulated with additional excipients to aid delivery and release. For example, in the context of dry powder formulations, the microparticles may be formulated with additional large carrier particles which aid the flow from the dry powder inhaler into the lung. Large carrier particles are known, and include lactose particles having a mass medium aerodynamic diameter of greater than 90 μm. Alternatively, the hydrophobic microparticles may be dispersed within a carrier material. For example, the

hydrophobic microparticles may be dispersed within a polysaccharide matrix, with the overall composition formulated as microparticles for direct delivery to the lung. The polysaccharide acts as a further barrier to the immediate release of the glycopyrrolate component. This may further aid the controlled release process. Suitable carrier materials will be apparent to the skilled person and include any pharmaceutically acceptable insoluble or soluble material, including polysaccharides. An example of a suitable polysaccharide is xantham gum.

The amount of the active agent to be used will depend on the usual factors, such as the potency of the agent, the nature and state of the condition to be treated, the state of the patient, etc. All these factors can be taken into account, and the relevant dose determined accordingly, by the skilled man.

The following study provides evidence on which this invention is based.

Study

Guinea pig tracheal strip preparations were pre-contracted with $5x10^{-6}$M carbachol for 10 minutes, to induce tone, before incubation with drug. The relaxation (% maximal of carbachol-induced tone) induced by the treatment is shown in Fig. 1; this drawing shows cumulative concentration-relaxation curves generated using a 5 minute dose-cycle. Relaxation is expressed as % maximal relaxation induced by $5x10^{-4}$M papaverine after completion of concentration-response curves. Maximal relaxation of time-matched control tissues is 3.5±1.9% after 50 minutes (35 minutes of dose-cycle).

EC_{50} values:-

	-Log EC_{50}	s.e.m	EC_{50} (M)	95% C.I.
glycopyrrolate	8.04	0.03	$9.23x10^{-9}$	$7.9x10^{-9} - 1.1x10^{-8}$
verapamil	4.56	0.35	$2.73x10^{-5}$	$1.8x10^{-5} - 4.0x10^{-5}$

Further experiments were conducted, to investigate the effect of pre-treatment with threshold concentrations on concentration-response curves to glycopyrrolate and verapamil. Again, guinea pig tracheal strip preparations were pre-contracted with $5x10^{-6}$M carbachol for 10 minutes before incubation with the pre-treatment drug. The relaxation (% maximal of carbachol-induced tone) induced by the pre-treatment is shown in Fig. 2, for the following groups.

Group	Pre-treatment	EC_{50} (M)	% Relaxation	DRC to
A	verapamil	$5x10^{-6}M$	6.6±0.5	glycopyrrolate
B	glycopyrrolate	$4x10^{-9}M$	6.5±0.8	verapamil
C	glycopyrrolate	$4x10^{-10}M$	-0.7±1.4	verapamil

In summary, the calcium antagonist verapamil is not a potent inhibitor of carbachol-induced bronchoconstriction in the guinea pig tracheal strip (EC_{50} $2.73x10^{-5}$). However, addition of a threshold dose of glycopyrrolate (a potent muscarinic antagonist) causes an almost 10-fold shift in EC_{50} for the drug (EC_{50} $5x10^{-6}$).

These results demonstrate the utility of calcium antagonists in combination with muscarinic antagonists in human respiratory disease where a bronchodilator may be necessary, e.g. asthma and COPD. It is also more general evidence of this invention.

CLAIMS

1. A product comprising an anti-muscarinic agent and a calcium channel blocker, for simultaneous, sequential or separate use in the treatment of a muscle tone disorder or a proliferative, inflammatory or secretory condition.

2. A product in the form of a medicament comprising an anti-muscarinic agent and a calcium channel blocker.

3. A product according to claim 1 or claim 2, in a form suitable for topical administration.

4. A product according to claim 1 or claim 2, in a form suitable for inhaled or systemic administration.

5. A product according to any preceding claim, wherein the anti-muscarinic agent and the calcium channel blocker are formulated to have different release rates.

6. A product according to any preceding claim, wherein the anti-muscarinic agent is glycopyrrolate.

7. A product according to any of claims 1 to 5, wherein the anti-muscarinic agent is tiotropium.

8. A product according to any of claims 1 to 7, wherein the calcium channel blocker is diltiazem.

9. A product according to any of claims 1 to 7, wherein the calcium channel blocker is verapamil.

10. A product according to any of claims 1 to 7, wherein the calcium channel blocker is a dihydropyridine drug.

11. A product according to any preceding claim, for the treatment of a proliferative, inflammatory or secretory condition.

12. Use of an anti-muscarinic agent for the manufacture of a medicament for use in the treatment of a muscle tone disorder or a proliferative, inflammatory or secretory condition in a patient undergoing treatment with a calcium channel blocker.

13. Use of a calcium channel blocker for the manufacture of a medicament for use in the treatment of a muscle tone disorder or a proliferative, inflammatory or secretory condition in a patient undergoing treatment with an anti-muscarinic agent.

14. Use according to claim 12 or claim 13, wherein the medicament is suitable for topical administration.

15. Use according to claim 12 or claim 13, wherein the medicament is suitable for inhaled administration.

16. Use according to any of claims 12 to 15, wherein the anti-muscarinic agent is glycopyrrolate.

17. Use according to any of claims 12 to 15, wherein the anti-muscarinic agent is tiotropium.

18. Use according to any of claims 12 to 17, wherein the calcium channel blocker is diltiazem.

19. Use product according to any of claims 12 to 17, wherein the calcium channel blocker is verapamil.

20. Use according to any of claims 12 to 17, wherein the calcium channel blocker is a dihydropyridine drug.

21. Use according to any of claims 12 to 20, wherein the condition is respiratory.

22. Use according to any of claims 12 to 21, for the treatment of a proliferative, inflammatory or secretory condition.

Figure 1

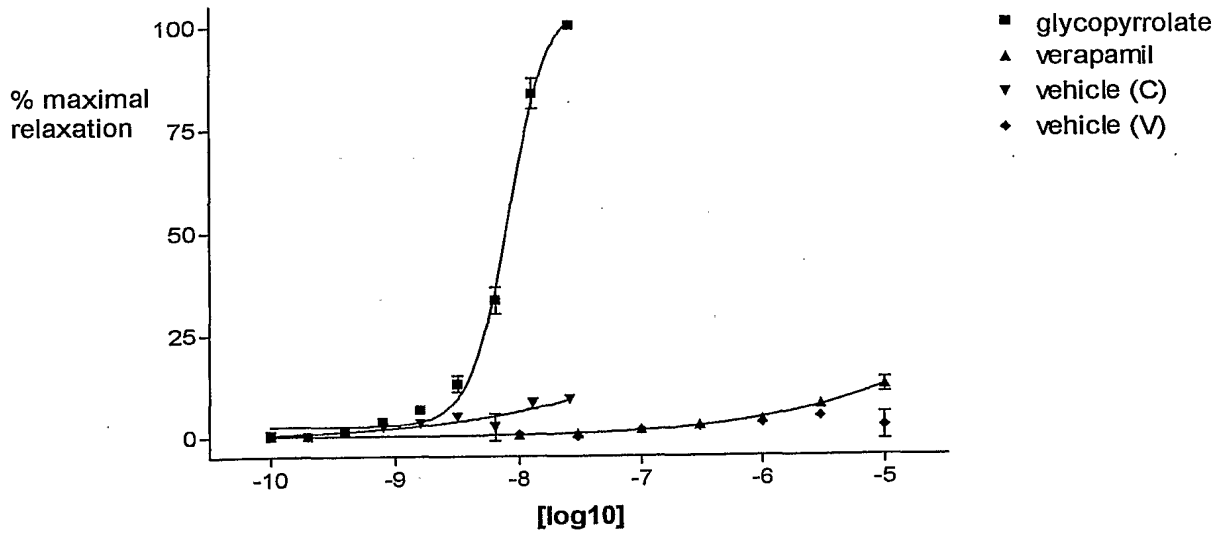
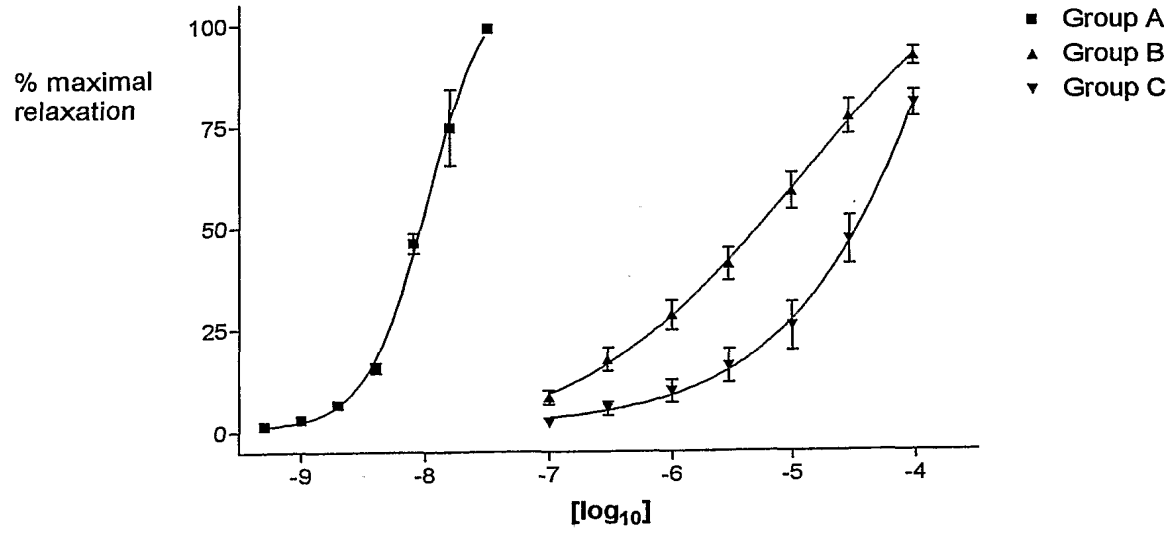


Figure 2



INTERNATIONAL SEARCH REPORT

PCT/GB 01/05450

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/40 A61K31/435 A61K31/55 A61K31/275 A61K9/00
A61P11/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, EPO-Internal, CHEM ABS Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 01 17479 A (ANDROSOLUTIONS INC) 15 March 2001 (2001-03-15) claims 1,9	1-22
A	WO 98 11888 A (AMERICAN HOME PROD) 26 March 1998 (1998-03-26) page 2, line 17-30; claims 1,2	1-22

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 April 2002	02/05/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Beyss, E

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-5, 10-15, 20-22 relate to an extremely large number of possible products. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the products claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds mentioned in claims 6-9 and 16-19.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

PCT/GB 01/05450

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0117479 A	15-03-2001	AU 7360000 A	10-04-2001
		AU 7360700 A	10-04-2001
		WO 0117479 A2	15-03-2001
		WO 0117480 A2	15-03-2001
WO 9811888 A	26-03-1998	AU 4421697 A	14-04-1998
		EP 0927034 A1	07-07-1999
		JP 2001502302 T	20-02-2001
		WO 9811888 A1	26-03-1998
		ZA 9708427 A	18-06-1999